



Return address: P.O. box 360, 3700 AJ Zeist, The Netherlands

William Stokes, D.V.M, D.A.C.L.A.M.  
Director, NICEATM/NIEHS  
P.O. Box 12233, K2-16  
Research Triangle, NC 27709



Utrechtseweg 48  
P.O. Box 360  
3700 AJ Zeist  
The Netherlands

[www.tno.nl](http://www.tno.nl)

T +31 30 694 41 44  
F +31 30 695 72 24  
[info-voeding@tno.nl](mailto:info-voeding@tno.nl)

**Date**  
May 14, 2009

**Our reference**  
TAP 2009-091/prm-hok

**E-mail**  
[ruud.woutersen@tno.nl](mailto:ruud.woutersen@tno.nl)

**Direct dialling**  
+31 30 694 45 03

**Direct fax**  
+31 30 694 49 86

**Attachments**

- Individual BRD data with TNO comments and modified BRD data
- Individual *in vivo* and ICE data compound TNO-28
- Letter TAP 2005 plus annexes

**Subject**  
ICE Report ICCVAM

Dear Dr. Stokes,

In reaction to the request for comments made public via the Federal Register notice, Vol. 74, No. 60, pages 14556-14557, March 31, 2009, the Dutch Research Organization TNO would like to forward the following comments and remarks concerning the ICE test method as described in the draft Proposed ICCVAM Test Method Recommendations: Evaluation of the Validation Status of Alternative Ocular Safety Testing Methods and Approaches and its Draft Background Review Document.

One specific comment on the draft BRD of the ICE is:

Page 4-1, lines 897-905. The text stating the data sets and presence of individual *in vivo* data are incorrect and incomplete. The Prinsen (2005) data set containing 49 compounds is not mentioned. Furthermore, both the Prinsen (1996) and Prinsen (2005) data set contained in total 94 compounds/ formulations which were all tested in parallel with the *in vivo* eye irritation test in rabbits (OECD no. 405) or the *in vivo* skin irritation/ corrosion test (OECD no. 404; in case of skin corrosive compounds/formulations). **All the individual rabbit data were made available to ICCVAM.** This section should be corrected and if it has implications on the classifications already assigned they should be corrected accordingly.

From the document it appeared that ICCVAM mainly focused on the performance of the ICE with respect to three main classification systems (EPA, GHS and EU). The basis for these classifications is the individual *in vitro* and *in vivo* data of the compounds available and ICCVAM's interpretation with respect to classification. It is rather alarming and disappointing to notice that the database contains so many gaps in the *in vivo* classifications, whereas sufficient *in vivo* data are available for such a purpose. Furthermore, we noticed that mistakes have been made in some of the classifications. To give an example, compound TNO-28 has been classified as R41, whereas the individual *in vivo* scores (see attachment) are even below the threshold for classification as an irritant according to the EU- and the GHS-criteria. ICCVAM's argument for the missing classifications is that the data do not comply with the four criteria set by ICCVAM. These cases mainly concern studies which were terminated earlier than 21 days after treatment or compounds lacking an *in vivo* eye irritation study because of proven *in vivo* skin corrosivity. We



Date  
May 14, 2009

Our reference  
TAP 2009-091/prm-hok

Page  
2/4

consider these arguments refutable, especially because of the significance of *in vitro* methods in general for replacing the *in vivo* rabbit eye test. Furthermore, by ICCVAM's description of their own selection criteria the possibility exists not to comply with them and still have the data used if a good reason can be provided. It is, therefore, a missed opportunity if one does not investigate into more detail the data that are available and we urge ICCVAM to perform the evaluation the data gaps after being filled in by expert judgment of the available data. Using TNO's long experience in eye irritation (*in vivo* and *in vitro* since 1983), the individual data of these cases were reviewed and commented by us (see attachment ICE BRD March 2006, Appendix D1, pages D-5 to D-9 with TNO comments and attachment with data gaps completed by TNO). We kindly request ICCVAM to take the data and comments into consideration for an additional evaluation.

Another important issue we would like to address is the fact that the scientific world has always considered the rabbit *in vivo* eye irritation test to produce highly variable results, making it almost impossible for any alternative method to be accepted. We would like to illustrate this with two examples that can be found in numerous articles presented by scientist in the field of validation. From the Proceedings of the first World Congress on Alternatives held in 1993, Chapter A.

**On Recognizing and Overcoming Barriers to the Acceptance of Alternative Methods** by Michael Balls and Julia Fentem, we would like to quote: "*In adequate analysis of data. It is very rare for any allowance to be made for the variability of the animal data, which are thus given a status which they do not deserve. They wrongly become the "true" values which the non-animal tests must struggle to reproduce. Also, insufficient allowance is made for the doubt which must be placed on values which fall within the barrier zones on both sides of category cut-off points. This is particular worrying when Cooper two-by-two way plots are used as a basis for establishing the sensitivity, specificity, predictivity and concordance of in vitro test data*".

From the publication in Toxicology *in vitro*, Volume 10 on Validation of alternative methods for toxicity testing (1996), the following: "*Computer simulations carried out by Bruner et al. have shown that, even if the alternative methods were perfectly reproducible (if their coefficients of variation were 0), the variability in the Draize scores alone would restrict the Pearson's correlation coefficients to the range 0.65 - 0.80 when the Draize scores are between 0 and 40 (typical of cosmetics ingredients)*". We would like to add that Draize scores between 0-40 in general represent the non to (mild) irritants.

The overall performance percentages obtained by the ICE (i.e. from all data sets combined) for non irritants were 66% (GHS), 78% (EPA) and 89% (EU).

Considering the in house TNO ICE data set of 1996 and 2005 which were tested in parallel with the *in vivo* rabbit eye test and thus excluding a considerable part of the *in vivo* variability, even a higher performance of 92-94% (EU) was obtained for non irritants.

The reason why ICCVAM does not assess the ICE at its true value originates from its observation that the ICE at least in one case classified a compound as non irritant whereas *in vivo* it was a corrosive/severe irritant. This case and other cases all pertained solids and in one case a very sticky antifouling paint (TNO-94). The issue of solids or sticky substances is of particular importance, because it is one of the main reasons for the long and unsuccessful history of validation of alternatives for eye irritation. This has all to do with the fact that the standard *in vivo* rabbit eye irritation/corrosion test (OECD guideline no. 405) has no standardized exposure regimen. We do not know how much of the compound



(solid, paste or liquid) and for how long the compound stays in contact with the eye; it can vary from minutes to hours. This kind of exposure is against all basic principles of toxicology testing, which is completely ignored in the evaluation by ICCVAM.

Further validation of alternatives would benefit tremendously if the **OECD guideline no. 405 would be modified to include a well-defined exposure regimen to the compound**. Moreover, the present exposure to solids can be considered highly unethical and cruel to the animals. Again we would like to quote the following from Michael Balls and Julia Fentem: "*To help bring this about (sic: more-rational attitudes to animal testing practices), the animal welfare movement must insist on the more-forceful application of animal protection laws such as Directive 86/609/EEC, which do not exclude regulatory animal testing from their Russell and Burch-inspired, three R's requirement that the use of animals be reduced, so that only the essential minimum number of animals are required, that the procedures necessarily applied to them be refined, so that they are more humane, and that they be replaced by non-animal methods wherever possible*".

Surely, ICCVAM together with ECVAM and other organizations dealing with alternatives and animal welfare, such as the NCA (Dutch Centre for Alternatives) could combine forces to initiate such a process. If the other initiative of ICCVAM on the "Use of Topical Anesthetics, Systemic Analgesics, and 46 Humane Endpoints in Ocular Toxicity Testing to Avoid or Minimize Pain and Distress" would lead to any modification of OECD no. 405, an excellent opportunity would arise to also address the exposure issue which would even contribute more to the minimizing of pain and distress in the animals.

Having the irrefutable fact that higher performance values will never be realized with the present rabbit eye irritation test as the "Golden Standard", an organotypic model such as **the ICE should be allowed in a tiered testing approach for the screening of non-irritants**. We feel that this statement is supported by our data and perspective given in this public comment. In the event that reanalysis does not acceptably resolve the situation of the false negatives (true or not by the Golden Standard) the following changes in the prediction model and/or applicability domain could be considered: 1) the ICE criteria for classification as non irritant could be set at the lowest combination of effects possible, i.e. no effect on the three parameters measured, and 2) solids could be excluded or tested additionally with a longer exposure period, although we strongly oppose to idea that the ICE should try to match the unrealistic exposure regimen of the Draize Eye test. For other purposes, TNO has obtained (confidential) data with longer exposure periods from which it appears that a 30 second application to solids provides useful additional information.

We would like to finish this letter with a message to ICCVAM by using a final quote from the article **On Recognizing and Overcoming Barriers to the Acceptance of Alternative Methods** by M. Balls and Julia Fentem:  
"All conceivable and practicable steps should be taken to make the formal acceptance and incorporation of non-animal toxicity test procedures into regulatory practice as smooth and rapid a process as is possible. The acceptance and incorporation process must not be rigid".

**Date**  
May 14, 2009

**Our reference**  
TAP 2009-091/prm-hok

**Page**  
3/4



We appreciate to have the opportunity to make these comments and kindly ask you to present them to the Expert Panel and general public before the meeting of 19-21 May 2009.

Yours faithfully,

A handwritten signature in black ink, appearing to read "Ruud A. Woutersen". It is written in a cursive style with a long horizontal line extending to the right.

Prof. Dr Ruud A. Woutersen  
Deputy Head of the Business Unit Toxicology and Applied Pharmacology, TNO  
Quality of Life  
Professor Translational Toxicology, Wageningen University

A handwritten signature in blue ink, appearing to read "M.K. Prinsen". It is written in a cursive style with a small flourish at the end.

M.K. Prinsen  
Toxicologist  
Project Leader *in vitro* and *in vivo* eye irritation  
Toxicology and Applied Pharmacology

**Date**  
May 14, 2009

**Our reference**  
TAP 2009-091/prm-hok

**Page**  
4/4

**In Vivo and In Vitro Comparison**  
**Sorted by Reference**

Substance/Product Name	CASRN	Concentration Tested	In Vitro Classification (GHS)	In Vivo Classification (GHS)	In Vitro Classification (EPA)	In Vivo Classification (EPA)	In Vitro Classification (EU)	In Vivo Classification (EU)	Reference
Acetone	67-64-1	undiluted	2A	2A	II	II	R36	R36	Balls et al. (1995)
Ammonium nitrate	6484-52-2	undiluted	2B	2B	III	III	NI	R36	Balls et al. (1995)
L-Aspartic acid	70-47-3	neat	2A	SCNM	II	SCNM	R36	SCNM	Balls et al. (1995)
Benzalkonium chloride (1%)	8001-54-5	1%	2A	1	II	I	R36	R41	Balls et al. (1995)
Benzalkonium chloride (5%)	8001-54-5	5%	1	1	I	I	R41	R41	Balls et al. (1995)
Benzalkonium chloride (10%)	8001-54-5	10%	1	1	I	I	R41	R41	Balls et al. (1995)
n-Butyl acetate	123-86-4	undiluted	2A	NI	II	III	R36	NI	Balls et al. (1995)
Gammabutyrolactone	96-48-0	undiluted	2A	2A	II	II	R36	R36	Balls et al. (1995)
Captan 90 concentrate	133-06-2	neat	2B	1	III	I	NI	R41	Balls et al. (1995)
4-Carboxybenzaldehyde	619-66-9	neat	NI	2A	IV	II	NI	R36	Balls et al. (1995)
Cetylpyridinium bromide (0.1%)	140-72-7	0.1%	2B	NI	III	III	NI	NI	Balls et al. (1995)
Cetylpyridinium bromide (6%)	140-72-7	6%	2A	1	II	SCNM	R36	R41	Balls et al. (1995)
Cetylpyridinium bromide (10%)	140-72-7	10%	2A	1	II	I	R36	R41	Balls et al. (1995)
Chlorhexidine	55-56-1	neat	1	1	I	SCNM	R41	SCNM	Balls et al. (1995)
Cyclohexanol	108-93-0	undiluted	1	1	I	1	R41	R41	Balls et al. (1995)
Dibenzoyl-L-tartaric acid	2743-38-6	neat	1	1	I	SCNM	R41	R41	Balls et al. (1995)
Dibenzylyl phosphate	1623-08-1	neat	2A/2B	2A	II/III	II	R36	R36	Balls et al. (1995)
2,6-Dichlorobenzoyl chloride	4659-45-4	undiluted	2A	2A	II	II	R36	SCNM	Balls et al. (1995)
2,2-Dimethylbutanoic acid	595-37-9	undiluted	1	SCNM	I	I	R41	SCNM	Balls et al. (1995)
2,5-Dimethylohexanediol	110-03-2	neat	2B	1	III	I	R36	R41	Balls et al. (1995)
Ethanol	64-17-5	undiluted	1	2A	I	III	R41	NI	Balls et al. (1995)
Ethyl acetate	141-78-6	undiluted	2A	NI	II	III	R36	NI	Balls et al. (1995)
2-Ethyl-1-hexanol	104-76-7	undiluted	2A	2A	II	II	R36	R36	Balls et al. (1995)
Ethyl-2-methylacetacetate	609-14-3	undiluted	2B	2B	III	III	NI	NI	Balls et al. (1995)
Ethyl trimethyl acetate	3938-95-2	undiluted	2B	NI	III	III	NI	NI	Balls et al. (1995)
Fomesafen	72128-02-0	neat	2B	NI	III	III	NI	NI	Balls et al. (1995)
Glycerol	56-81-5	undiluted	2B	NI	III	IV	NI	NI	Balls et al. (1995)
n-Hexanol	111-27-3	undiluted	1	2A	I	II	R41	R36	Balls et al. (1995)
Imidazole	288-32-4	neat	1	1	I	I	R41	R41	Balls et al. (1995)
Isobutanol	78-83-1	undiluted	1	2A	I	II	R41	R36	Balls et al. (1995)
Isopropanol	67-63-0	undiluted	1	2A	I	III	R41	SCNM	Balls et al. (1995)
Maneb	12427-38-2	neat	NI	SCNM	IV	III	NI	SCNM	Balls et al. (1995)
Methyl acetate	79-20-9	undiluted	1	2A	I	II	R41	R36	Balls et al. (1995)
Methyl cyanoacetate	105-34-0	undiluted	NI	2A	IV	II	NI	R36	Balls et al. (1995)
Methyleclopentane	96-37-7	undiluted	NI	NI	IV	III	NI	NI	Balls et al. (1995)
Methyl ethyl ketone	78-93-3	undiluted	1	2A	I	III	R41	R36	Balls et al. (1995)
Methyl isobutyl ketone	108-10-1	undiluted	2A	NI	II	III	R36	NI	Balls et al. (1995)
1-Naphthaleneacetic acid	86-87-3	neat	2B	1	III	I	R36	SCNM	Balls et al. (1995)
1-Naphthaleneacetic acid, sodium salt	61-31-4	neat	1	1	I	I	R41	R41	Balls et al. (1995)
n-Octanol	111-87-5	undiluted	2A	2B	II	II	R36	R36	Balls et al. (1995)
Parafluoraniline	371-40-4	undiluted	1	SCNM	I	SCNM	R41	SCNM	Balls et al. (1995)
Polyethylene glycol 400	25322-68-3	undiluted	2B	NI	III	IV	R36	NI	Balls et al. (1995)
Potassium cyanate	590-28-3	neat	2B	SCNM	III	SCNM	R36	SCNM	Balls et al. (1995)
Promethazine HCl	58-33-3	neat	1	1	I	I	R41	R41	Balls et al. (1995)

**In Vivo and In Vitro Comparison**  
**Sorted by Reference**

Substance/Product Name	CASRN	Concentration Tested	In Vitro Classification (GHS)	In Vivo Classification (GHS)	In Vitro Classification (EPA)	In Vivo Classification (EPA)	In Vitro Classification (EU)	In Vivo Classification (EU)	Reference
Pyridine	110-86-1	undiluted	1	1	I	I	R41	R41	Balls et al. (1995)
Quinacrine	69-05-6	neat	2B	1	III	I	NI	R41	Balls et al. (1995)
Sodium hydroxide (1%)	1310-73-2	1%	2A	2B	II	III	R36	R36	Balls et al. (1995)
Sodium hydroxide (10%)	1310-73-2	10%	1	1	I	I	R41	R41	Balls et al. (1995)
Sodium lauryl sulfate (3%)	151-21-3	3%	2B	NI	III	III	NI	NI	Balls et al. (1995)
Sodium lauryl sulfate (15%)	151-21-3	15%	2B	1	III	I	R36	R36	Balls et al. (1995)
Sodium oxalate	62-76-0	neat	2B	1	III	I	NI	R41	Balls et al. (1995)
Sodium perborate, 4H <sub>2</sub> O	10486-00-7	neat	2B	1	III	I	NI	R41	Balls et al. (1995)
Tetraaminopyrimidine sulfate	5392-28-9	neat	2B	NI	III	III	NI	NI	Balls et al. (1995)
Toluene	108-88-3	undiluted	2A	NI	II	III	R36	NI	Balls et al. (1995)
Trichloroacetic acid (3%)	76-03-9	3%	2A	NI	II	III	R36	NI	Balls et al. (1995)
Trichloroacetic acid (30%)	76-03-9	30%	1	1	I	I	R41	R41	Balls et al. (1995)
Triton X-100 (5%)	9002-93-1	5%	2A	2A	II	III	R36	NI	Balls et al. (1995)
Triton X-100 (10%)	9002-93-1	10%	2A/2B	1	II/III	II	R36	R41	Balls et al. (1995)
Tween 20	9005-64-5	undiluted	2B	NI	III	III	NI	NI	Balls et al. (1995)
TNO-01 (Formulation-1)	n.p.	undiluted	NI	NI	IV	IV	NI	NI	Prinsen (1996)
TNO-02 (Formulation-2)	n.p.	undiluted	2A	2A	II	II	R36	R36	Prinsen (1996)
TNO-03 (Pesticide-1)	n.p.	undiluted	NI	NI	IV	III	NI	NI	Prinsen (1996)
TNO-04 (Detergent-1)	n.p.	undiluted	2B	2A	III	III	NI	NI	Prinsen (1996)
TNO-05 (Silicone powder-1)	n.p.	undiluted	NI	NI	IV	IV	NI	NI	Prinsen (1996)
TNO-06 (Lubricant)	n.p.	undiluted	NI	NI	IV	IV	NI	NI	Prinsen (1996)
TNO-07 (Ink-1)	n.p.	undiluted	NI	NI	IV	IV	NI	NI	Prinsen (1996)
TNO-08 (Ink-2)	n.p.	undiluted	NI	NI	IV	IV	NI	NI	Prinsen (1996)
TNO-09 (Paint)	n.p.	undiluted	NI	NI	IV	II	NI	NI	Prinsen (1996)
TNO-10 (Silicone powder-2)	n.p.	undiluted	NI	NI	IV	IV	NI	NI	Prinsen (1996)
TNO-11 (Sodium p-styrene sulfonate)	2695-37-6	undiluted	2A	SCNM	II	SCNM	R36	SCNM	Prinsen (1996)
TNO-12 (Formulation-3)	n.p.	undiluted	2A	NI	II	SCNM	R36	R36	Prinsen (1996)
TNO-13 (Pesticide-2)	n.p.	undiluted	NI	NI	IV	IV	NI	NI	Prinsen (1996)
TNO-14 (Polydisaccharide)	n.p.	14.5%	NI	NI	IV	IV	NI	NI	Prinsen (1996)
TNO-15 (Polydisaccharide)	n.p.	50%	NI	NI	IV	IV	NI	NI	Prinsen (1996)
TNO-16 (Liquid nylon product)	n.p.	undiluted	NI	NI	IV	IV	NI	NI	Prinsen (1996)
TNO-17 (Solvent-1)	n.p.	undiluted	NI	NI	IV	IV	NI	NI	Prinsen (1996)
TNO-18 (Solvent-2)	n.p.	undiluted	NI	NI	IV	IV	NI	NI	Prinsen (1996)
TNO-19 (Solvent-3)	n.p.	undiluted	NI	NI	IV	IV	NI	NI	Prinsen (1996)
TNO-20 (Solvent-4)	n.p.	undiluted	NI	NI	IV	IV	NI	NI	Prinsen (1996)
TNO-21 (Solvent-5)	n.p.	undiluted	NI	NI	IV	IV	NI	NI	Prinsen (1996)
TNO-22 (Solvent-6)	n.p.	undiluted	NI	NI	IV	IV	NI	NI	Prinsen (1996)
TNO-23 (Solvent-7)	n.p.	undiluted	NI	NI	IV	IV	NI	NI	Prinsen (1996)
TNO-24 (Solvent-8)	n.p.	undiluted	NI	NI	IV	IV	NI	NI	Prinsen (1996)
TNO-25 (Solvent-9)	n.p.	undiluted	NI	NI	IV	IV	NI	NI	Prinsen (1996)
TNO-26 (Ink-3)	n.p.	undiluted	NI	NI	IV	IV	NI	NI	Prinsen (1996)
TNO-27 (Thermal paper coating-1)	n.p.	undiluted	2B	2B	III	III	NI	NI	Prinsen (1996)
TNO-28 (Toilet cleaner-1)	n.p.	undiluted	2B	1	III	I	NI	R41	Prinsen (1996)
TNO-29 (Toilet cleaner-2)	n.p.	undiluted	2B	2A	III	III	NI	R36	Prinsen (1996)

**In Vivo and In Vitro Comparison**  
**Sorted by Reference**

Substance/Product Name	CASRN	Concentration Tested	In Vitro Classification (GHS)	In Vivo Classification (GHS)	In Vitro Classification (EPA)	In Vivo Classification (EPA)	In Vitro Classification (EU)	In Vivo Classification (EU)	Reference
TNO-30 (Pesticide-3)	n.p.	undiluted	2B	NI	III	IV	NI	NI	Prinsen (1996)
TNO-31 (Sulfur)	7704-34-9	undiluted	NI	NI	IV	III	NI	NI	Prinsen (1996)
TNO-32 (Ink-4)	n.p.	undiluted	2B	NI	III	IV	NI	NI	Prinsen (1996)
TNO-33 (Thermal paper coating-2)	n.p.	undiluted	2B	NI	III	IV	NI	NI	Prinsen (1996)
TNO-34 (Detergent-2)	n.p.	undiluted	1	SCNM	I	SCNM	R41	SCNM	Prinsen (1996)
TNO-35 (Propyl-lactate)	616-09-1	undiluted	1	1	I	I	R41	R41	Prinsen (1996)
TNO-36 (Ethylhexyl lactate)	6283-86-9	undiluted	2A	SCNM	II	II	R36	SCNM	Prinsen (1996)
TNO-37 (Pesticide-4)	n.p.	undiluted	2B	2B	III	III	NI	NI	Prinsen (1996)
TNO-38 (Solvent-10)	n.p.	undiluted	NI	NI	IV	IV	NI	NI	Prinsen (1996)
TNO-39 (Detergent-3)	n.p.	undiluted	NI	NI	IV	IV	NI	NI	Prinsen (1996)
TNO-40 (Glycolbromoacetate form.)	n.p.	undiluted	1	-	I	-	R41	R41 (SC)	Prinsen (1996)
TNO-41 (Amidosulfonic acid)	5329-14-6	undiluted	1	-	I	-	R41	R41 (SC)	Prinsen (1996)
TNO-42 (Glycolbromoacetate)	3785-34-0	85%	1	-	I	-	R41	R41 (SC)	Prinsen (1996)
TNO-43 (Monobromoacetic acid)	79-08-3	undiluted	1	-	I	-	R41	R41 (SC)	Prinsen (1996)
TNO-44 (Didecyldimethylammoniumchloride (23% in propyl glycol))	7173-51-5	23%	1	-	I	-	R41	R41 (SC)	Prinsen (1996)
Cetylpyridinium bromide (6%)	—	undiluted	1	1	I	SCNM	R41	R41	Prinsen (2000)
cyclohexylamino-functional PMS	—	undiluted	2A	-	II	-	R36	R36	Prinsen (2000)
decamethylcyclopentasiloxane	—	undiluted	NI	-	NI	-	NI	NI	Prinsen (2000)
Triton X-500 (5%)	—	undiluted	2B	-	III	-	NI	R36	Prinsen (2000)
TNO-45	n.p.	undiluted	NI	NI	NI	IV	NI	NI	Prinsen (2005)
TNO-46	n.p.	undiluted	NI	NI	NI	IV	NI	NI	Prinsen (2005)
TNO-47	n.p.	undiluted	NI	NI	NI	IV	NI	NI	Prinsen (2005)
TNO-48	n.p.	undiluted	2A	-	II	-	R36	R41 (SC)	Prinsen (2005)
TNO-49	n.p.	undiluted	1	-	I	-	R41	R41 (SC)	Prinsen (2005)
TNO-50	n.p.	undiluted	1	-	I	-	R41	R41 (SC)	Prinsen (2005)
TNO-51	n.p.	undiluted	1	-	I	-	R41	R41 (SC)	Prinsen (2005)
TNO-52	n.p.	undiluted	2B	2A	III	III	NI	R36	Prinsen (2005)
TNO-53	n.p.	undiluted	NI	NI	IV	NI	NI	NI	Prinsen (2005)
TNO-54	n.p.	undiluted	2B	2B	III	III	NI	NI	Prinsen (2005)
TNO-55	n.p.	undiluted	2B	2A	III	III	R36	R36	Prinsen (2005)
TNO-56	n.p.	undiluted	2B	2B	III	III	R36	NI	Prinsen (2005)
TNO-57	n.p.	undiluted	2B	NI	III	IV	NI	NI	Prinsen (2005)
TNO-58	n.p.	undiluted	NI	NI	NI	IV	NI	NI	Prinsen (2005)
TNO-59	n.p.	undiluted	NI	NI	NI	IV	NI	NI	Prinsen (2005)
TNO-60	n.p.	undiluted	NI	NI	NI	IV	NI	NI	Prinsen (2005)
TNO-61	n.p.	undiluted	NI	NI	NI	IV	NI	NI	Prinsen (2005)
TNO-62	n.p.	undiluted	2B	NI	III	III	R36	NI	Prinsen (2005)
TNO-63	n.p.	undiluted	NI	NI	IV	III	NI	NI	Prinsen (2005)
TNO-64	n.p.	undiluted	2B	NI	III	IV	NI	NI	Prinsen (2005)
TNO-65	n.p.	undiluted	NI	NI	NI	IV	NI	NI	Prinsen (2005)

**In Vivo and In Vitro Comparison**  
**Sorted by Reference**

Substance/Product Name	CASRN	Concentration Tested	In Vitro Classification (GHS)	In Vivo Classification (GHS)	In Vitro Classification (EPA)	In Vivo Classification (EPA)	In Vitro Classification (EU)	In Vivo Classification (EU)	Reference
TNO-66	n.p.	undiluted	NI	NI	NI	IV	NI	NI	Prinsen (2005)
TNO-67	n.p.	undiluted	2B	NI	III	IV	NI	NI	Prinsen (2005)
TNO-68	n.p.	undiluted	2A	2A	II	II	R36	R36	Prinsen (2005)
TNO-69	n.p.	50%	NI	NI	NI	IV	NI	NI	Prinsen (2005)
TNO-70	n.p.	undiluted	2A	2A	II	III	R36	R36	Prinsen (2005)
TNO-71	n.p.	undiluted	2B	NI	III	IV	NI	NI	Prinsen (2005)
TNO-72	n.p.	undiluted	NI	NI	NI	IV	NI	NI	Prinsen (2005)
TNO-73	n.p.	undiluted	1	2A	I	II	R41	R36	Prinsen (2005)
TNO-74	n.p.	undiluted	NI	NI	IV	III	NI	NI	Prinsen (2005)
TNO-75	n.p.	undiluted	NI	NI	NI	IV	NI	NI	Prinsen (2005)
TNO-76	n.p.	undiluted	NI	NI	NI	IV	NI	NI	Prinsen (2005)
TNO-77	n.p.	undiluted	2B	NI	III	IV	NI	NI	Prinsen (2005)
TNO-78	n.p.	undiluted	2B	2B	III	III	NI	NI	Prinsen (2005)
TNO-79	n.p.	undiluted	2B	NI	III	IV	NI	NI	Prinsen (2005)
TNO-80	n.p.	undiluted	NI	NI	NI	IV	NI	NI	Prinsen (2005)
TNO-81	n.p.	undiluted	NI	NI	NI	IV	NI	NI	Prinsen (2005)
TNO-82	n.p.	undiluted	NI	NI	NI	IV	NI	NI	Prinsen (2005)
TNO-83	n.p.	undiluted	2B	2B	III	III	NI	R36	Prinsen (2005)
TNO-84	n.p.	undiluted	2B	NI	III	IV	NI	NI	Prinsen (2005)
TNO-85	n.p.	undiluted	2B	1	III	I	R36	R41	Prinsen (2005)
TNO-86	n.p.	undiluted	2B	NI	III	IV	NI	NI	Prinsen (2005)
TNO-87	n.p.	undiluted	2B	NI	III	IV	NI	NI	Prinsen (2005)
TNO-88	n.p.	undiluted	NI	NI	NI	IV	NI	NI	Prinsen (2005)
TNO-89	n.p.	undiluted	NI	NI	NI	IV	NI	NI	Prinsen (2005)
TNO-90	n.p.	undiluted	NI	NI	NI	IV	NI	NI	Prinsen (2005)
TNO-91	n.p.	undiluted	NI	NI	NI	IV	NI	NI	Prinsen (2005)
TNO-92	n.p.	undiluted	2B	1	III	I	R36	R41	Prinsen (2005)
TNO-93	n.p.	undiluted	2A	1	II	I	R36	R41	Prinsen (2005)
TNO-94	n.p.	undiluted	NI	1	NI	I	NI	R41	Prinsen (2005)
1-Butanol	71-36-3	undiluted	1	2A	I	II	R41	R41	Prinsen and Koëter (1993)
2-Butoxyethyl acetate	112-07-2	undiluted	2B	-	III	-	NI	NI	Prinsen and Koëter (1993)
2-Methoxyethanol	109-86-4	undiluted	2A	-	II	-	R36	R36	Prinsen and Koëter (1993)
Acetaldehyde	75-07-0	undiluted	2A	-	II	-	R36	R36	Prinsen and Koëter (1993)
Acetic acid	64-19-7	10%	1	1	I	I	R41	R41	Prinsen and Koëter (1993)
Benzalkonium chloride (100%)	8001-54-5	undiluted	1	1	I	I	R41	R41	Prinsen and Koëter (1993)
Brij 35	9002-92-0	undiluted	NI	-	IV	-	NI	NI	Prinsen and Koëter (1993)
Chloroform	67-66-3	undiluted	2A	-	II	-	R36	R36	Prinsen and Koëter (1993)
Dibutyltin dichloride	683-18-1	undiluted	1	-	I	-	R41	R41	Prinsen and Koëter (1993)
Dimethyl sulfoxide	67-68-5	undiluted	NI	2B	IV	III	NI	NI	Prinsen and Koëter (1993)
Glycerol	56-81-5	undiluted	NI	NI	IV	IV	NI	NI	Prinsen and Koëter (1993)
Mercury (II) chloride	7487-94-7	undiluted	1	-	I	-	R41	R41	Prinsen and Koëter (1993)
n-Hexane	110-54-3	undiluted	NI	NI	IV	IV	NI	NI	Prinsen and Koëter (1993)
Silver (I) nitrate	7761-88-8	3%	2B	-	III	-	NI	NI	Prinsen and Koëter (1993)
Sodium dodecyl sulfate	151-21-3	undiluted	2B	-	III	-	R41	R41	Prinsen and Koëter (1993)

**In Vivo and In Vitro Comparison**  
**Sorted by Reference**

March 2006

Substance/Product Name	CASRN	Concentration Tested	<i>In Vitro</i> Classification (GHS)	<i>In Vivo</i> Classification (GHS)	<i>In Vitro</i> Classification (EPA)	<i>In Vivo</i> Classification (EPA)	<i>In Vitro</i> Classification (EU)	<i>In Vivo</i> Classification (EU)	Reference
Sodium fluorescein	518-47-8	20%	NI	-	IV	-	NI	NI	Prinsen and Koëter (1993)
Sodium hydroxide	1310-73-2	1%	1	1	I	I	R41	R41	Prinsen and Koëter (1993)
Toluene	108-88-3	undiluted	2B	2B	III	III	NI	NI	Prinsen and Koëter (1993)
Triacetin	102-76-1	undiluted	NI	NI	IV	IV	NI	NI	Prinsen and Koëter (1993)
Tributyltin chloride	1461-22-9	undiluted	1	-	I	-	R41	R41	Prinsen and Koëter (1993)
Triethanolamine	102-71-6	undiluted	2B	NI	III	III	NI	NI	Prinsen and Koëter (1993)

SCNM - Study criteria not met

SC - Classification assigned on the basis of skin corrosion assay

Substance/Product Name	CASRN	Concentration Tested	In Vitro Classification (GHS)	In Vivo Classification (GHS)	In Vitro Classification (EPA)	In Vivo Classification (EPA)	In Vitro Classification (EU)	In Vivo Classification (EU)	Reference
Acetone	67-64-1	undiluted	2A	2A	II	II	R36	R36	Balls et al. (1995)
Ammonium nitrate	6484-52-2	undiluted	2B	2B	III	III	NI	R36	Balls et al. (1995)
L-Aspartic acid	70-47-3	neat	2A	SCNM	II	SCNM	R36	SCNM	Balls et al. (1995)
Benzalkonium chloride (1%)	8001-54-5	1%	2A	1	II	I	R36	R41	Balls et al. (1995)
Benzalkonium chloride (5%)	8001-54-5	5%	1	1	I	I	R41	R41	Balls et al. (1995)
Benzalkonium chloride (10%)	8001-54-5	10%	1	1	I	I	R41	R41	Balls et al. (1995)
n-Butyl acetate	123-86-4	undiluted	2A	NI	II	III	R36	NI	Balls et al. (1995)
Gammabutyrolactone	96-48-0	undiluted	2A	2A	II	II	R36	R36	Balls et al. (1995)
Captan 90 concentrate	133-06-2	neat	2B	1	III	I	NI	R41	Balls et al. (1995)
4-Carboxybenzaldehyde	619-66-9	neat	NI	2A	IV	II	NI	R36	Balls et al. (1995)
Cetylpyridinium bromide (0.1%)	140-72-7	0.1%	2B	NI	III	III	NI	NI	Balls et al. (1995)
Cetylpyridinium bromide (6%)	140-72-7	6%	2A	1	II	SCNM	R36	R41	Balls et al. (1995)
Cetylpyridinium bromide (10%)	140-72-7	10%	2A	1	II	I	R36	R41	Balls et al. (1995)
Chlorhexidine	55-56-1	neat	1	1	I	SCNM	R41	SCNM	Balls et al. (1995)
Cyclohexanol	108-93-0	undiluted	1	1	I	I	R41	R41	Balls et al. (1995)
Dibenzoyl-L-tartaric acid	2743-38-6	neat	1	1	I	SCNM	R41	R41	Balls et al. (1995)
Dibenzyl phosphate	1623-08-1	neat	2A/2B	2A	II/III	II	R36	R36	Balls et al. (1995)
2,6-Dichlorobenzoyl chloride	4659-45-4	undiluted	2A	2A	II	II	R36	SCNM	Balls et al. (1995)
2,2-Dimethylbutanoic acid	595-37-9	undiluted	1	1	I	I	R41	R41	Balls et al. (1995)
2,5-Dimethoxyhexanediol	110-03-2	neat	2B	1	III	I	R36	R41	Balls et al. (1995)
Ethanol	64-17-5	undiluted	1	2A	I	III	R41	NI	Balls et al. (1995)
Ethyl acetate	141-78-6	undiluted	2A	NI	II	III	R36	NI	Balls et al. (1995)
2-Ethyl-1-hexanol	104-76-7	undiluted	2A	2A	II	II	R36	R36	Balls et al. (1995)
Ethyl-2-methylacetoacetate	609-14-3	undiluted	2B	2B	III	III	NI	NI	Balls et al. (1995)
Ethyl trimethyl acetate	3938-95-2	undiluted	2B	NI	III	III	NI	NI	Balls et al. (1995)
Fomesafen	72128-02-0	neat	2B	NI	III	III	NI	NI	Balls et al. (1995)
Glycerol	56-81-5	undiluted	2B	NI	III	IV	NI	NI	Balls et al. (1995)
n-Hexanol	111-27-3	undiluted	1	2A	I	II	R41	R36	Balls et al. (1995)
Imidazole	288-32-4	neat	1	1	I	I	R41	R41	Balls et al. (1995)
Isobutanol	78-83-1	undiluted	1	2A	I	II	R41	R36	Balls et al. (1995)
Isopropanol	67-63-0	undiluted	1	2A	I	III	R41	SCNM	Balls et al. (1995)
Maneb	12427-38-2	neat	NI	SCNM	IV	III	NI	SCNM	Balls et al. (1995)
Methyl acetate	79-20-9	undiluted	1	2A	I	II	R41	R36	Balls et al. (1995)
Methyl cyanoacetate	105-34-0	undiluted	NI	2A	IV	II	NI	R36	Balls et al. (1995)
Methylcyclopentane	96-37-7	undiluted	NI	NI	IV	III	NI	NI	Balls et al. (1995)
Methyl ethyl ketone	78-93-3	undiluted	1	2A	I	III	R41	R36	Balls et al. (1995)
Methyl isobutyl ketone	108-10-1	undiluted	2A	NI	II	III	R36	NI	Balls et al. (1995)
1-Naphthaleneacetic acid	86-87-3	neat	2B	1	III	I	R36	SCNM	Balls et al. (1995)
1-Naphthaleneacetic acid, sodium salt	61-31-4	neat	1	1	I	I	R41	R41	Balls et al. (1995)
n-Octanol	111-87-5	undiluted	2A	2B	II	II	R36	R36	Balls et al. (1995)
Parafluoraniline	371-40-4	undiluted	1	1	I	SCNM	R41	R41	Balls et al. (1995)
Polyethylene glycol 400	25322-68-3	undiluted	2B	NI	III	IV	R36	NI	Balls et al. (1995)
Potassium cyanate	590-28-3	neat	2B	SCNM	III	SCNM	R36	SCNM	Balls et al. (1995)
Promethazine HCl	58-33-3	neat	1	1	I	I	R41	R41	Balls et al. (1995)

Substance/Product Name	CASRN	Concentration Tested	In Vitro Classification (GHS)	In Vivo Classification (GHS)	In Vitro Classification (EPA)	In Vivo Classification (EPA)	In Vitro Classification (EU)	In Vivo Classification (EU)	Reference
Pyridine	110-86-1	undiluted	1	1	I	I	R41	R41	Balls et al. (1995)
Quinacrine	69-05-6	neat	2B	1	III	I	NI	R41	Balls et al. (1995)
Sodium hydroxide (1%)	1310-73-2	1%	2A	2B	II	III	R36	R36	Balls et al. (1995)
Sodium hydroxide (10%)	1310-73-2	10%	1	1	I	I	R41	R41	Balls et al. (1995)
Sodium lauryl sulfate (3%)	151-21-3	3%	2B	NI	III	III	NI	NI	Balls et al. (1995)
Sodium lauryl sulfate (15%)	151-21-3	15%	2B	2A	III	I	R36	R36	Balls et al. (1995)
Sodium oxalate	62-76-0	neat	2B	1	III	I	NI	R41	Balls et al. (1995)
Sodium perborate, 4H2O	10486-00-7	neat	2B	1	III	I	NI	R41	Balls et al. (1995)
Tetraaminopyrimidine sulfate	5392-28-9	neat	2B	NI	III	III	NI	NI	Balls et al. (1995)
Toluene	108-88-3	undiluted	2A	NI	II	III	R36	NI	Balls et al. (1995)
Trichloroacetic acid (3%)	76-03-9	3%	2A	NI	II	III	R36	NI	Balls et al. (1995)
Trichloroacetic acid (30%)	76-03-9	30%	1	1	I	I	R41	R41	Balls et al. (1995)
Triton X-100 (5%)	9002-93-1	5%	2A	2A	II	III	R36	NI	Balls et al. (1995)
Triton X-100 (10%)	9002-93-1	10%	2A/2B	1	II/III	II	R36	R41	Balls et al. (1995)
Tween 20	9005-64-5	undiluted	2B	NI	III	III	NI	NI	Balls et al. (1995)

Substance/Product Name	CASRN	Concentration Tested	In Vitro Classification (GHS)	In Vivo Classification (GHS)	In Vitro Classification (EPA)	In Vivo Classification (EPA)	In Vitro Classification (EU)	In Vivo Classification (EU)	Reference
TNO-01 (Formulation-1)	n.p.	undiluted	NI	NI	IV	IV	NI	NI	Prinsen (1996)
TNO-02 (Formulation-2)	n.p.	undiluted	2A	2A	II	II	R36	R36	Prinsen (1996)
TNO-03 (Pesticide-1)	n.p.	undiluted	NI	NI	IV	III	NI	NI	Prinsen (1996)
TNO-04 (Detergent-1)	n.p.	undiluted	2B	2A	III	III	NI	NI	Prinsen (1996)
TNO-05 (Silicone powder-1)	n.p.	undiluted	NI	NI	IV	IV	NI	NI	Prinsen (1996)
TNO-06 (Lubricant)	n.p.	undiluted	NI	NI	IV	IV	NI	NI	Prinsen (1996)
TNO-07 (Ink-1)	n.p.	undiluted	NI	NI	IV	IV	NI	NI	Prinsen (1996)
TNO-08 (Ink-2)	n.p.	undiluted	NI	NI	IV	IV	NI	NI	Prinsen (1996)
TNO-09 (Paint)	n.p.	undiluted	NI	NI	IV	II	NI	NI	Prinsen (1996)
TNO-10 (Silicone powder-2)	n.p.	undiluted	NI	NI	IV	IV	NI	NI	Prinsen (1996)
TNO-11 (Sodium p-styrene sulfonate)	2695-37-6	undiluted	2A	2A	II	SCNM	R36	R36	Prinsen (1996)
TNO-12 (Formulation-3)	n.p.	undiluted	2A / I	I	II	SCNM	R36/R41	R41	Prinsen (1996)
TNO-13 (Pesticide-2)	n.p.	undiluted	NI	NI	IV	IV	NI	NI	Prinsen (1996)
TNO-14 (Polydisaccharide)	n.p.	14.5%	NI	NI	IV	IV	NI	NI	Prinsen (1996)
TNO-15 (Polydisaccharide)	n.p.	50%	NI	NI	IV	IV	NI	NI	Prinsen (1996)
TNO-16 (Liquid nylon product)	n.p.	undiluted	NI	NI	IV	IV	NI	NI	Prinsen (1996)
TNO-17 (Solvent-1)	n.p.	undiluted	NI	NI	IV	IV	NI	NI	Prinsen (1996)
TNO-18 (Solvent-2)	n.p.	undiluted	NI	NI	IV	IV	NI	NI	Prinsen (1996)
TNO-19 (Solvent-3)	n.p.	undiluted	NI	NI	IV	IV	NI	NI	Prinsen (1996)
TNO-20 (Solvent-4)	n.p.	undiluted	NI	NI	IV	IV	NI	NI	Prinsen (1996)
TNO-21 (Solvent-5)	n.p.	undiluted	NI	NI	IV	IV	NI	NI	Prinsen (1996)
TNO-22 (Solvent-6)	n.p.	undiluted	NI	NI	IV	IV	NI	NI	Prinsen (1996)
TNO-23 (Solvent-7)	n.p.	undiluted	NI	NI	IV	IV	NI	NI	Prinsen (1996)
TNO-24 (Solvent-8)	n.p.	undiluted	NI	NI	IV	IV	NI	NI	Prinsen (1996)
TNO-25 (Solvent-9)	n.p.	undiluted	NI	NI	IV	IV	NI	NI	Prinsen (1996)
TNO-26 (Ink-3)	n.p.	undiluted	NI	NI	IV	IV	NI	NI	Prinsen (1996)
TNO-27 (Thermal paper coating-1)	n.p.	undiluted	2B	2B	III	III	NI	NI	Prinsen (1996)
TNO-28 (Toilet cleaner-1)	n.p.	undiluted	2B	2A	III	I	NI	NI/R36	Prinsen (1996)
TNO-29 (Toilet cleaner-2)	n.p.	undiluted	2B	2A	III	III	NI	NI	Prinsen (1996)

Substance/Product Name	CASRN	Concentration Tested	In Vitro Classification (GHS)	In Vivo Classification (GHS)	In Vitro Classification (EPA)	In Vivo Classification (EPA)	In Vitro Classification (EU)	In Vivo Classification (EU)	Reference
TNO-30 (Pesticide-3)	n.p.	undiluted	2B	NI	III	IV	NI	NI	Prinsen (1996)
TNO-31 (Sulfur)	7704-34-9	undiluted	NI	NI	IV	III	NI	NI	Prinsen (1996)
TNO-32 (Ink-4)	n.p.	undiluted	2B	NI	III	IV	NI	NI	Prinsen (1996)
TNO-33 (Thermal paper coating-2)	n.p.	undiluted	NI	NI	III	IV	NI	NI	Prinsen (1996)
TNO-34 (Detergent-2)	n.p.	undiluted	1	1	I	I	SCNM	R41	R41
TNO-35 (Propyl-lactate)	616-09-1	undiluted	1	1	I	I	R41	R41	Prinsen (1996)
TNO-36 (Ethylhexyl lactate)	6283-86-9	undiluted	2A	2A	II	II	R36	R36	Prinsen (1996)
TNO-37 (Pesticide-4)	n.p.	undiluted	2B	2B	III	III	NI	NI	Prinsen (1996)
TNO-38 (Solvent-10)	n.p.	undiluted	NI	NI	IV	IV	NI	NI	Prinsen (1996)
TNO-39 (Detergent-3)	n.p.	undiluted	NI	NI	IV	IV	NI	NI	Prinsen (1996)
TNO-40 (Glycolbromoacetate form.)	n.p.	undiluted	1	1 (SC)	I	-	R41	R41 (SC)	Prinsen (1996)
TNO-41 (Amidosulfonic acid)	5329-14-6	undiluted	1	1 (SC)	I	-	R41	R41 (SC)	Prinsen (1996)
TNO-42 (Glycolbromoacetate)	3785-34-0	85%	1	1 (SC)	I	-	R41	R41 (SC)	Prinsen (1996)
TNO-43 (Monobromoacetic acid)	79-08-3	undiluted	1	1 (SC)	I	-	R41	R41 (SC)	Prinsen (1996)
TNO-44 (Didecyldimethylammoniumchloride (23% in propyl glycol))	7173-51-5	23%	1	-	I	-	R41	R41 (SC)	Prinsen (1996)
Cetylpyridinium bromide (6%)	□	undiluted	1	1	I	SCNM	R41	R41	Prinsen (2000)
cyclohexylamino-functional PMS	□	undiluted	2A	-	II	-	R36	R36	Prinsen (2000)
decamethylcyclopentasiloxane	□	undiluted	NI	-	NI	-	NI	NI	Prinsen (2000)
Triton X-500 (5%)	□	undiluted	2B	-	III	-	NI	R36	Prinsen (2000)
TNO-45	n.p.	undiluted	NI	NI	NI	IV	NI	NI	Prinsen (2005)
TNO-46	n.p.	undiluted	NI	NI	NI	IV	NI	NI	Prinsen (2005)
TNO-47	n.p.	undiluted	NI	NI	NI	IV	NI	NI	Prinsen (2005)
TNO-48	n.p.	undiluted	2A/1	1 (SC)	II	-	R36 /R41	R41 (SC)	Prinsen (2005)
TNO-49	n.p.	undiluted	1	1 (SC)	I	-	R41	R41 (SC)	Prinsen (2005)
TNO-50	n.p.	undiluted	1	1 (SC)	I	-	R41	R41 (SC)	Prinsen (2005)
TNO-51	n.p.	undiluted	1	1 (SC)	I	-	R41	R41 (SC)	Prinsen (2005)
TNO-52	n.p.	undiluted	2B	2A	III	III	NI /R36	R36	Prinsen (2005)
TNO-53	n.p.	undiluted	NI	NI	IV	-	NI	NI	Prinsen (2005)
TNO-54	n.p.	undiluted	2B	2B	III	III	NI	NI	Prinsen (2005)
TNO-55	n.p.	undiluted	2B	2A	III	III	R36	R36	Prinsen (2005)
TNO-56	n.p.	undiluted	2B	2B	III	III	R36	NI (R36)	Prinsen (2005)
TNO-57	n.p.	undiluted	2B	NI	III	IV	NI	NI	Prinsen (2005)
TNO-58	n.p.	undiluted	NI	NI	NI	IV	NI	NI	Prinsen (2005)
TNO-59	n.p.	undiluted	NI	NI	NI	IV	NI	NI	Prinsen (2005)
TNO-60	n.p.	undiluted	NI	NI	NI	IV	NI	NI	Prinsen (2005)
TNO-61	n.p.	undiluted	NI	NI	NI	IV	NI	NI	Prinsen (2005)
TNO-62	n.p.	undiluted	2B	NI	III	III	R36	NI	Prinsen (2005)
TNO-63	n.p.	undiluted	NI	NI	IV	III	NI	NI	Prinsen (2005)
TNO-64	n.p.	undiluted	2B	NI	III	IV	NI	NI	Prinsen (2005)
TNO-65	n.p.	undiluted	NI	NI	NI	IV	NI	NI	Prinsen (2005)

Substance/Product Name	CASRN	Concentration Tested	In Vitro Classification (GHS)	In Vivo Classification (GHS)	In Vitro Classification (EPA)	In Vivo Classification (EPA)	In Vitro Classification (EU)	In Vivo Classification (EU)	Reference
TNO-66	n.p.	undiluted	NI	NI	NI	IV	NI	NI	Prinsen (2005)
TNO-67	n.p.	undiluted	2B	NI	III	IV	NI	NI	Prinsen (2005)
TNO-68	n.p.	undiluted	2A	2A	II	II	R36	R36	Prinsen (2005)
TNO-69	n.p.	50%	NI	NI	NI	IV	NI	NI	Prinsen (2005)
TNO-70	n.p.	undiluted	2A	2A	II	III	R36	R36	Prinsen (2005)
TNO-71	n.p.	undiluted	2B	NI	III	IV	NI	NI	Prinsen (2005)
TNO-72	n.p.	undiluted	NI	NI	NI	IV	NI	NI	Prinsen (2005)
TNO-73	n.p.	undiluted	1	2A(1)	I	II	R41	R36(R41)	Prinsen (2005)
TNO-74	n.p.	undiluted	NI	NI	IV	III	NI	NI	Prinsen (2005)
TNO-75	n.p.	undiluted	NI	NI	NI	IV	NI	NI	Prinsen (2005)
TNO-76	n.p.	undiluted	NI	NI	NI	IV	NI	NI	Prinsen (2005)
TNO-77	n.p.	undiluted	2B	NI	III	IV	NI	NI	Prinsen (2005)
TNO-78	n.p.	undiluted	2B	2B	III	III	NI	NI	Prinsen (2005)
TNO-79	n.p.	undiluted	2B	NI	III	IV	NI	NI	Prinsen (2005)
TNO-80	n.p.	undiluted	NI	NI	NI	IV	NI	NI	Prinsen (2005)
TNO-81	n.p.	undiluted	NI	NI	NI	IV	NI	NI	Prinsen (2005)
TNO-82	n.p.	undiluted	NI	NI	NI	IV	NI	NI	Prinsen (2005)
TNO-83	n.p.	undiluted	2B	2B	III	III	NI/R36	R36	Prinsen (2005)
TNO-84	n.p.	undiluted	2B	NI	III	IV	NI	NI	Prinsen (2005)
TNO-85	n.p.	undiluted	2B	1	III	I	R36	R41	Prinsen (2005)
TNO-86	n.p.	undiluted	2B	NI	III	IV	NI	NI	Prinsen (2005)
TNO-87	n.p.	undiluted	2B	NI	III	IV	NI	NI	Prinsen (2005)
TNO-88	n.p.	undiluted	NI	NI	NI	IV	NI	NI	Prinsen (2005)
TNO-89	n.p.	undiluted	NI	NI	NI	IV	NI	NI	Prinsen (2005)
TNO-90	n.p.	undiluted	NI	NI	NI	IV	NI	NI	Prinsen (2005)
TNO-91	n.p.	undiluted	NI	NI	NI	IV	NI	NI	Prinsen (2005)
TNO-92	n.p.	undiluted	2B	1	III	I	R36	R41*	Prinsen (2005)
TNO-93	n.p.	undiluted	2A	1	II	I	R36	R41	Prinsen (2005)
TNO-94	n.p.	undiluted	NI	1	NI	I	NI	R41**	Prinsen (2005)
1-Butanol	71-36-3	undiluted	1	2A	I	II	R41	R41	Prinsen and Koëter (1993)
2-Butoxyethyl acetate	112-07-2	undiluted	2B	-	III	-	NI	NI	Prinsen and Koëter (1993)
2-Methoxyethanol	109-86-4	undiluted	2A	-	II	-	R36	R36	Prinsen and Koëter (1993)
Acetaldehyde	75-07-0	undiluted	2A	-	II	-	R36	R36	Prinsen and Koëter (1993)
Acetic acid	64-19-7	10%	1	1	I	I	R41	R41	Prinsen and Koëter (1993)
Benzalkonium chloride (100%)	8001-54-5	undiluted	1	1	I	I	R41	R41	Prinsen and Koëter (1993)
Brij 35	9002-92-0	undiluted	NI	-	IV	-	NI	NI	Prinsen and Koëter (1993)
Chloroform	67-66-3	undiluted	2A	-	II	-	R36	R36	Prinsen and Koëter (1993)
Dibutyltin dichloride	683-18-1	undiluted	1	-	I	-	R41	R41	Prinsen and Koëter (1993)
Dimethyl sulfoxide	67-68-5	undiluted	NI	2B	IV	III	NI	NI	Prinsen and Koëter (1993)
Glycerol	56-81-5	undiluted	NI	NI	IV	IV	NI	NI	Prinsen and Koëter (1993)
Mercury (II) chloride	7487-94-7	undiluted	1	-	I	-	R41	R41	Prinsen and Koëter (1993)
n-Hexane	110-54-3	undiluted	NI	NI	IV	IV	NI	NI	Prinsen and Koëter (1993)
Silver (I) nitrate	7761-88-8	3%	2B	-	III	-	NI	NI	Prinsen and Koëter (1993)
Sodium dodecyl sulfate	151-21-3	undiluted	2B	-	III	-	R41	R41	Prinsen and Koëter (1993)

Substance/Product Name	CASRN	Concentratio n Tested	<i>In Vitro</i> <i>Classification</i> (GHS)	<i>In Vivo</i> <i>Classification</i> (GHS)	<i>In Vitro</i> <i>Classification</i> (EPA)	<i>In Vivo</i> <i>Classification</i> (EPA)	<i>In Vitro</i> <i>Classification</i> (EU)	<i>In Vivo</i> <i>Classification</i> (EU)	Reference
Sodium fluorescein	518-47-8	20%	NI	-	IV	-	NI	NI	Prinsen and Koëter (1993)
Sodium hydroxide	1310-73-2	1%	1	1	I	I	R41	R41	Prinsen and Koëter (1993)
Toluene	108-88-3	undiluted	2B	2B	III	III	NI	NI	Prinsen and Koëter (1993)
Triacetin	102-76-1	undiluted	NI	NI	IV	IV	NI	NI	Prinsen and Koëter (1993)
Tributyltin chloride	1461-22-9	undiluted	1	-	I	-	R41	R41	Prinsen and Koëter (1993)
Triethanolamine	102-71-6	undiluted	2B	NI	III	III	NI	NI	Prinsen and Koëter (1993)

SCNM - Study criteria not met

SC - Classification assigned on the basis of skin corrosion assay

\* powder with entrapment in cul-de-sac

\*\* paint adhering to the cornea

V 93.592

Mean scores EU: December  
1993Page  
10

Compound TNO-20

no 492 Redness 0.7  
no 532 1.7 1.3  
no 537 2.3 2.0swelling  
eye effects  
reversible

EC-criteria only mentions

that the observation period should be sufficient to evaluate  
fully reversibility or irrevers. of the eye effects

Table 1 - Individual scores awarded to the ocular lesions elicited by

rabbit number	corneal opacity	iris effects	conjunctivae redness	conjunctivae chemosis	ocular discharge
---------------	-----------------	--------------	----------------------	-----------------------	------------------

⇒ Compound is NI (but borderline)  
like in the IEE

after one hour

492	0	1	1	2	2	$5 + 10 = 15$
532	0	1	1	2	2	$5 + 10 = 15$
537	0	1	<u>1</u>	2	2	$5 + 10 = 15$
			<u>3</u>	<u>6</u>	<u>6</u>	<u>15</u>
						<u>30</u>
						<u>45</u>

after 24 hours

492	0	0	1	1	0	<u>4</u>
532	0	0	2	2	1	<u>10</u>
537	0	0	<u>1</u>	2	1	<u>8</u>
			<u>4</u>	<u>5</u>	<u>2</u>	<u>22</u>

after 48 hours

492	0	0	1	1	0	<u>4</u>
532	0	0	2	1	0	<u>0</u>
537	0	0	<u>3<sup>1</sup></u>	<u>2</u>	0	<u>10</u>
			<u>6</u>	<u>4</u>		<u>20</u>

after 72 hours

492	0	0	0	0	0	<u>0</u>
532	0	0	1	1	0	<u>4</u>
537	0	0	<u>3<sup>1</sup></u>	<u>2</u>	0	<u>10</u>
			<u>4</u>	<u>3</u>		<u>14</u>

after 7 days

492	0	0	0	0	0	<u>0</u>
532	0	0	0	0	0	<u>0</u>
537	0	0	<u>3<sup>1</sup></u>	2	1	<u>12</u>
			<u>4</u>	<u>2</u>		<u>12</u>

after 14 and 21 days

537	0	0	2	1	<u>1<sup>2</sup></u>	<u>8</u>
-----	---	---	---	---	----------------------	----------

after 28 days

537	0	0	0	0	0	<u>0</u>
-----	---	---	---	---	---	----------

<sup>1</sup> = ischemic necrosis on the nictitating membrane<sup>2</sup> = white ocular discharge $\sum 61$ 

The scores are explained in the appendix on the next page

W $\sum 121$

(28)

V 93.593

May  
1994Page  
12

Table 1 - Individual values for corneal swelling, corneal opacity and fluorescein retention values of the control and test eyes obtained with ......

Eye no.	Corneal Swelling	Corneal Opacity	Fluorescein Retention
after 30 minutes			
1 <sup>1</sup>	6.8	0.0	2.0
2	6.7	0.0	1.0
3	9.5	0.0	2.0
4	10.0	0.0	1.0
5	6.3	0.0	1.0
control	3.0	0.0	0.0
after 75 minutes			
1	6.8	1.0	
2	8.3	0.5	
3	6.3	0.5	
4	8.3	0.0	
5	7.9	0.0	
control	-3.0	0.0	
after 120 minutes			
1	10.2	1.0	
2	10.0	0.5	
3	11.1	0.5	
4	11.7	0.0	
5	9.5	0.0	
control	-1.5	0.0	
after 180 minutes			
1	11.9	1.0	
2	10.0	0.5	
3	9.5	0.5	
4	15.0	0.0	
5	9.5	0.0	
control	-3.0	0.0	
after 240 minutes			
1	13.6	1.0	
2	10.0	1.0	
3	9.5	0.5	
4	13.3	0.5	
5	14.3	0.0	
control	-3.0	0.0	

<sup>1</sup> eye no. 1 also showed some slight loosening of epithelial cells of the cornea

Table 2 - Mean values for corneal swelling, corneal opacity and fluorescein retention values of the test eyes treated with ... and the irritancy categories based on the maximum scores, and the final EC-classification

Time intervals	Corneal		Fluorescein
	Swelling	Opacity	Retention
30	7.9 (0.8)	0.0 (0.0)	1.4 (0.2)
75	7.5 (0.4)	0.4 (0.2)	
120	10.5 (0.4)	0.4 (0.2)	
180	11.2 (1.1)	0.4 (0.2)	
240	12.1 (1.0)	0.8 (0.1)	

parameter	maximum score	category
Corneal swelling	12.1	II
Corneal opacity	0.8	II
Fluorescein retention	1.4	II

(EC-)classification: NOT IRRITANT

BORDERLINE CASE BETWEEN NON-IRRITATING AND IRRITATING

between brackets = standard error of the mean



Return address: Postbus 360, 3700 AJ, Zeist, The Netherlands

NTP/NICEATM  
NIEHS  
Attn. Dr. Raymond Tice  
P.O. Box 12233, MD EC-17  
Research Triangle Park, NC 27709  
**USA – BY AIRMAIL**

Toxicology and Applied  
Pharmacology  
Location Zeist  
Utrechtseweg 48  
P.O. Box 360  
3700 AJ Zeist  
The Netherlands

[www.tno.nl](http://www.tno.nl)  
T +31 30 694 41 44  
F +31 30 695 72 24  
[infofood@voeding.tno.nl](mailto:infofood@voeding.tno.nl)

**Subject**

Expert Panel Report ICCVAM/NICEATM

Dear Sir,

In reaction to the request for comments made public via the Federal Register notice, Volume 70, No. 142, Tuesday, July 26, 2005/Notices, 43149, the Dutch Research Organization TNO would like to forward the following comments and remarks concerning the ICE test method described in the Addendum to "*In Vitro* Ocular Toxicity Draft Background Review Documents".

The inclusion of the additional data, forwarded by TNO beginning of this year, is highly appreciated. Because TNO has a longstanding experience with the screening of severe eye irritants for contract research, the additional data was forwarded to substantiate this particular application of the ICE. The data contained the full set of chemicals and/or formulations that was tested *in vitro* and *in vivo* over a period of several years. As we have experienced over the last 20 years of contract *in vivo* eye and skin irritation testing, about 10% of the compounds consist of eye/skin corrosives, of which almost all were screened by the ICE as indeed severely eye irritating. Therefore, we found it rather peculiar that eight severe eye irritating compounds were excluded from the reanalysis on the basis that insufficient *in vivo* data was provided to classify the compound according to the GHS classification system. All these compounds were corrosive in the *in vivo* skin irritation test, which was performed after the outcome of the ICE. The individual *in vivo* skin irritation data of these compounds were provided to ICCVAM. In full agreement with the guidelines, TNO decided to waive the *in vivo* eye irritation test in rabbits in these cases.

As the main purpose of the ICE (and any other *in vitro* eye irritation method) in contract research is to prevent severe irritants to be tested in the Draize eye test, it is rather paradoxical to see that all these actual cases of correctly identified severe eye irritants are not taken into account for the evaluation of this method.

**Date**  
August 8, 2005

**Our reference**  
TAP-2005

**Contact**  
Dr. R.A. Woutersen

**E-mail**  
[woutersen@voeding.tno.nl](mailto:woutersen@voeding.tno.nl)

**Direct dialling**  
+31 30 694 4503

**Direct fax**  
+31 30 696 02 64

The Standard Conditions for Research Instructions given to TNO, as filed at the Registry of the District Court and the Chamber of Commerce in The Hague shall apply to all instructions given to TNO; the Standard Conditions will be sent on request.

**Attachments**  
Article TIV (pdf : 4 pages)  
ECETOC data (3 pages)



In contrast to what is taken by ICCVAM as the reason for exclusion, the GHS criteria clearly mentions “corrosive to skin” as one of the criteria for class I “irreversible eye effects”. For the EPA classification, I cannot imagine that a skin corrosive is not assumed to be a category I compound for eye irritation. Furthermore, two compounds from the EC/HO validation study study, i.e. *p*-fluoroaniline and 2,2-dimethyl butanoic acid, were excluded from the reanalysis. Both compounds were identified by the management of the EC/HO validation study (Balls et al., 1995) as R41 severely eye irritating on the basis of the individual *in vivo* data (ECETOC, Technical document no. 48: 2, June 1998; data attached). These two compounds were also correctly identified by the ICE and most other *in vitro* methods participating.

The *in vivo* classification was based on sound scientific judgment and it is unclear on which basis ICCVAM refuted this expert judgment. The probable reason for ICCVAM to exclude the compounds, may be that a 21-day observation period was not completed, is inadequate and, if so, demonstrates insufficient knowledge of the eye irritation process in rabbits. Moreover, the guidelines (at the time of testing) specified that the observation period should be long enough to evaluate the reversibility or irreversibility of the lesions.

The six rabbits treated with 2,2-dimethyl butanoic acid still showed slight to severe corneal opacity and neovascularization of the cornea at 14 days after treatment. Clearly, these lesions are not reversible within a 21-day observation period.

The same applies to *p*-fluoroaniline, causing moderate to severe corneal opacity and iritis score 2 (highest score possible; no reaction to light, haemorrhage, gross destruction). The test was terminated on day 3, which is in agreement with the current guidelines which mention that animals may be humanely sacrificed if the severity of the effects is considered too high.

On the basis of the above, TNO strongly requests ICCVAM to revise the present analyses with respect to the screening of severe irritants by inclusion of these ten cases. We have also concern about the fact that the data of the various studies performed with the ICE in different time periods are pooled for analyses and that the outcome of the individual studies is not discussed individually. Success or failure of *in vitro* methods has much to do with the setting in which the method is used and the way the *in vivo* data was obtained. Therefore, we advise ICCVAM to also mention and comment the successful use and strategy of the ICE by TNO for screening severe irritants.

With respect to the proposed Candidate substances (appendix V-A1), TNO would like to express reservations about the usefulness/appropriateness of such a list, containing only summary data. By now, after all the validation studies already carried out, we know that quite a different approach for validation is needed, including a meticulous test substance selection. With respect to this issue, we would like to draw your attention to the discussion article attached to this letter and which is currently in press in Toxicology In Vitro. This article deals, among other things, with the problem of using historical *in vivo* eye data for validation of *in vitro* test systems. We hope this article will contribute to the discussion concerning the validation process, i.e. not starting a new validation process before dealing with the basic issues of the *in vivo* test.

**Date**  
August 8, 2005

**Our reference**  
TAP-2005

**Page**  
2/3

**Attachments**  
Article TIV (pdf : 4 pages)  
ECETOC data (3 pages)



We are looking forward ICCVAM's official reaction to our comments. In the mean time, TNO is, as always, available for additional information and discussion, if needed. In that respect, we were rather disappointed that we were not asked to comment on the above mentioned issue and, moreover, we were not informed of the availability of the reanalysis on the ICCVAM website.

Yours faithfully,

A handwritten signature in black ink, appearing to read "Ruud A. Woutersen".

Dr Ruud A. Woutersen  
Head of the Business Unit Toxicology and Applied Pharmacology, TNO Quality of Life

**Date**  
August 8, 2005

**Our reference**  
TAP-2005

**Page**  
3/3

**Attachments**  
Article TIV (pdf : 4 pages)  
ECETOC data (3 pages)

*p*-Fluoroaniline

Animal No.	4	Observation period (days)											
		1 h	4 h	1	2	3	4	7	9	10	12	14	21
Cornea	Opacity A	1	2	1	1	2	-	-	-	-	-	-	-
	Area involved B	2	4	4	4	4	-	-	-	-	-	-	-
	(AxB) x 5	10	40	20	20	40	-	-	-	-	-	-	-
Iris	C	0	1	0	0	1	-	-	-	-	-	-	-
	C x 5	0	5	0	0	5	-	-	-	-	-	-	-
Conjunctiva	Redness D	1	2	3	3	3	-	-	-	-	-	-	-
	Chemosis E	2	3	2	2	3	-	-	-	-	-	-	-
	Discharge F	1	1	1	2	2	-	-	-	-	-	-	-
	(D+E+F) x 2	8	12	12	14	16	-	-	-	-	-	-	-
<i>Total</i>		18	57	32	34	61	-	-	-	-	-	-	-

Animal No.	5	Observation period (days)											
		1 h	4 h	1	2	3	4	7	9	10	12	14	21
Cornea	Opacity A	1	1	1	1	3	-	-	-	-	-	-	-
	Area involved B	2	4	4	4	4	-	-	-	-	-	-	-
	(AxB) x 5	10	20	20	20	60	-	-	-	-	-	-	-
Iris	C	0	0	0	0	2	-	-	-	-	-	-	-
	C x 5	0	0	0	0	10	-	-	-	-	-	-	-
Conjunctiva	Redness D	1	2	2	2	3	-	-	-	-	-	-	-
	Chemosis E	2	3	2	2	2	-	-	-	-	-	-	-
	Discharge F	1	1	2	2	2	-	-	-	-	-	-	-
	(D+E+F) x 2	8	12	12	12	14	-	-	-	-	-	-	-
<i>Total</i>		18	32	32	32	84	-	-	-	-	-	-	-

Animal No.	6	Observation period (days)											
		1 h	4 h	1	2	3	4	7	9	10	12	14	21
Cornea	Opacity A	1	1	1	1	2	-	-	-	-	-	-	-
	Area involved B	2	4	4	4	4	-	-	-	-	-	-	-
	(AxB) x 5	10	20	20	20	40	-	-	-	-	-	-	-
Iris	C	0	0	0	0	2	-	-	-	-	-	-	-
	C x 5	0	0	0	0	10	-	-	-	-	-	-	-
Conjunctiva	Redness D	2	2	2	3	3	-	-	-	-	-	-	-
	Chemosis E	2	3	2	2	3	-	-	-	-	-	-	-
	Discharge F	1	1	1	2	3	-	-	-	-	-	-	-
	(D+E+F) x 2	10	12	10	14	18	-	-	-	-	-	-	-
<i>Total</i>		20	32	30	34	68	-	-	-	-	-	-	-

MMAS (Modified Maximum Average Score)  $(52+66+88+61+84+68) / 6 = 69.8$

NB Test terminated at 3 days

opacity  $28/18 = 1.6$

## 2,2-Dimethyl Butanoic Acid

Source	Aldrich			Concentration tested	100 %
Specification				Volume tested	0.1 ml
CAS No.	595-37-9			No. of rabbits	6
Purity	96 %				
Product No.	D15,260-9				

			Observation period (days)											
Animal No.	1		1 h	4 h	1	2	3	4	7	9	10	12	14	21
Cornea	Opacity	A	1	-	1	1	2	-	2	-	-	-	-	2 <sup>a</sup>
	Area involved	B	4	-	4	4	4	-	4	-	-	-	-	4
	(AxB) x 5		20	-	20	20	40	-	40	-	-	-	-	40
Iris		C	0	-	0	1	1	-	1	-	-	-	-	1
	C x 5		0	-	0	5	5	-	5	-	-	-	-	5
Conjunctiva	Redness	D	2	-	2	2	2	-	2	-	-	-	-	0
	Chemosis	E	1	-	1	0	0	-	0	-	-	-	-	0
	Discharge	F	1	-	1	1	2	-	1	-	-	-	-	0
	(D+E+F) x 2		8	-	8	6	8	-	6	-	-	-	-	0
	<b>Total</b>		<b>28</b>	-	<b>28</b>	<b>31</b>	<b>53</b>	-	<b>51</b>	-	-	-	-	<b>45</b>

			Observation period (days)											
Animal No.	2		1 h	4 h	1	2	3	4	7	9	10	12	14	21
Cornea	Opacity	A	2	-	2	1	1	-	2	-	-	-	-	1 <sup>a</sup>
	Area involved	B	4	-	4	4	4	-	4	-	-	-	-	3
	(AxB) x 5		40	-	40	20	20	-	40	-	-	-	-	15
Iris		C	1	-	0	0	0	-	0	-	-	-	-	0
	C x 5		5	-	0	0	0	-	0	-	-	-	-	0
Conjunctiva	Redness	D	2	-	2	2	2	-	2	-	-	-	-	0
	Chemosis	E	1	-	1	0	0	-	0	-	-	-	-	0
	Discharge	F	1	-	0	1	1	-	0	-	-	-	-	0
	(D+E+F) x 2		8	-	6	6	6	-	4	-	-	-	-	0
	<b>Total</b>		<b>53</b>	-	<b>46</b>	<b>26</b>	<b>26</b>	-	<b>44</b>	-	-	-	-	<b>15</b>

			Observation period (days)											
Animal No.	3		1 h	4 h	1	2	3	4	7	9	10	12	14	21
Cornea	Opacity	A	1	-	1	1	1	-	1	-	-	-	-	1
	Area involved	B	4	-	4	4	4	-	4	-	-	-	-	2
	(AxB) x 5		20	-	20	20	20	-	20	-	-	-	-	10
Iris		C	0	-	0	0	0	-	0	-	-	-	-	0
	C x 5		0	-	0	0	0	-	0	-	-	-	-	0
Conjunctiva	Redness	D	2	-	2	2	2	-	1	-	-	-	-	0
	Chemosis	E	3	-	1	0	0	-	0	-	-	-	-	0
	Discharge	F	1	-	0	1	1	-	0	-	-	-	-	0
	(D+E+F) x 2		12	-	6	6	6	-	2	-	-	-	-	0
	<b>Total</b>		<b>32</b>	-	<b>26</b>	<b>26</b>	<b>26</b>	-	<b>22</b>	-	-	-	-	<b>10</b>

## 2,2-Dimethyl Butanoic Acid

Animal No.		4	Observation period (days)											
			1 h	4 h	1	2	3	4	7	9	10	12	14	21
Cornea	Opacity	A	2	-	2	2	2	-	2	-	-	-	1 <sup>a</sup>	-
	Area involved	B	4	-	4	4	4	-	4	-	-	-	4	-
	(AxB) x 5		40	-	40	40	40	-	40	-	-	-	20	-
Iris		C	1	-	1	1	1	-	1	-	-	-	0	-
	C x 5		5	-	5	5	5	-	5	-	-	-	0	-
Conjunctiva	Redness	D	2	-	2	2	2	-	2	-	-	-	0	-
	Chemosis	E	2	-	1	1	1	-	0	-	-	-	0	-
	Discharge	F	1	-	1	2	2	-	1	-	-	-	0	-
	(D+E+F) x 2		10	-	8	10	10	-	6	-	-	-	0	-
<b>Total</b>			<b>55</b>	-	<b>53</b>	<b>55</b>	<b>55</b>	-	<b>51</b>	-	-	-	<b>20</b>	-

Animal No.		5	Observation period (days)											
			1 h	4 h	1	2	3	4	7	9	10	12	14	21
Cornea	Opacity	A	1	-	1	1	2	-	3 <sup>a</sup>	-	-	-	3 <sup>a</sup>	-
	Area involved	B	4	-	4	4	4	-	4	-	-	-	4	-
	(AxB) x 5		20	-	20	20	40	-	60	-	-	-	60	-
Iris		C	0	-	0	0	1	-	1	-	-	-	2	-
	C x 5		0	-	0	0	5	-	5	-	-	-	10	-
Conjunctiva	Redness	D	2	-	2	2	2	-	2	-	-	-	1	-
	Chemosis	E	2	-	1	0	1	-	1	-	-	-	0	-
	Discharge	F	1	-	1	0	2	-	1	-	-	-	0	-
	(D+E+F) x 2		10	-	8	4	10	-	8	-	-	-	2	-
<b>Total</b>			<b>30</b>	-	<b>28</b>	<b>24</b>	<b>55</b>	-	<b>73</b>	-	-	-	<b>72</b>	-

Animal No.		6	Observation period (days)											
			1 h	4 h	1	2	3	4	7	9	10	12	14	21
Cornea	Opacity	A	2	-	2	2	2	-	1	-	-	-	2 <sup>a</sup>	-
	Area involved	B	4	-	4	4	4	-	4	-	-	-	4	-
	(AxB) x 5		40	-	40	40	40	-	20	-	-	-	40	-
Iris		C	1	-	1	1	1	-	0	-	-	-	0	-
	C x 5		5	-	5	5	5	-	0	-	-	-	0	-
Conjunctiva	Redness	D	2	-	2	2	2	-	1	-	-	-	0	-
	Chemosis	E	2	-	2	1	1	-	0	-	-	-	0	-
	Discharge	F	1	-	1	1	1	-	0	-	-	-	0	-
	(D+E+F) x 2		10	-	10	8	8	-	2	-	-	-	0	-
<b>Total</b>			<b>55</b>	-	<b>55</b>	<b>53</b>	<b>53</b>	-	<b>22</b>	-	-	-	<b>40</b>	-

MMAS (Modified Maximum Average Score)  $(53+26+26+55+55+53) / 6 = 44.7$

<sup>a</sup> Corneal vascularisation

NB Test Terminated at 14 days

**Sodium Lauryl Sulphate**

Source Specification	Sigma	Concentration tested	15.0 %
CAS No.	151-21-3	Volume tested	0.1 ml
Purity	98 %	No. of rabbits	6
Product No.	L-4509		

Animal No.	1	Observation period (days)											
		1 h	4 h	1	2	3	4	7	9	10	12	14	21
Cornea	Opacity A	-	-	2	2	1	-	0	-	0	-	-	-
	Area involved B	-	-	4	3	2	-	0	-	0	-	-	-
	(AxB) x 5	-	-	40	30	10	-	0	-	0	-	-	-
Iris	C	-	-	0	0	0	-	1	-	0	-	-	-
		-	-	0	0	0	-	5	-	0	-	-	-
Conjunctiva	Redness D	-	-	2	2	2	-	2	-	0	-	-	-
	Chemosis E	-	-	2	1	1	-	1	-	0	-	-	-
	Discharge F	-	-	3	2	1	-	0	-	0	-	-	-
	(D+E+F) x 2	-	-	14	10	8	-	6	-	0	-	-	-
<b>Total</b>		-	-	<b>54</b>	<b>40</b>	<b>18</b>	-	<b>11</b>	-	<b>0</b>	-	-	-

Animal No.	2	Observation period (days)											
		1 h	4 h	1	2	3	4	7	9	10	12	14	21
Cornea	Opacity A	-	-	2	2	1	-	0	-	-	-	-	-
	Area involved B	-	-	3	2	1	-	0	-	-	-	-	-
	(AxB) x 5	-	-	30	20	5	-	0	-	-	-	-	-
Iris	C	-	-	1	1	0	-	0	-	-	-	-	-
		-	-	5	5	0	-	0	-	-	-	-	-
Conjunctiva	Redness D	-	-	2	2	1	-	0	-	-	-	-	-
	Chemosis E	-	-	2	1	1	-	0	-	-	-	-	-
	Discharge F	-	-	2	1	1	-	0	-	-	-	-	-
	(D+E+F) x 2	-	-	12	8	6	-	0	-	-	-	-	-
<b>Total</b>		-	-	<b>47</b>	<b>33</b>	<b>11</b>	-	<b>0</b>	-	-	-	-	-

Animal No.	3	Observation period (days)											
		1 h	4 h	1	2	3	4	7	9	10	12	14	21
Cornea	Opacity A	-	-	2	2	1	-	0	-	0	-	-	-
	Area involved B	-	-	4	2	1	-	0	-	0	-	-	-
	(AxB) x 5	-	-	40	20	5	-	0	-	0	-	-	-
Iris	C	-	-	2	2	1	-	0	-	0	-	-	-
		-	-	10	10	5	-	0	-	0	-	-	-
Conjunctiva	Redness D	-	-	2	2	2	-	0	-	0	-	-	-
	Chemosis E	-	-	3	2	2	-	1	-	0	-	-	-
	Discharge F	-	-	3	2	1	-	0	-	0	-	-	-
	(D+E+F) x 2	-	-	16	12	10	-	2	-	0	-	-	-
<b>Total</b>		-	-	<b>66</b>	<b>42</b>	<b>20</b>	-	<b>2</b>	-	<b>0</b>	-	-	-

**Sodium Lauryl Sulphate**

Animal No.	4		Observation period (days)											
			1 h	4 h	1	2	3	4	7	9	10	12	14	21
Cornea	Opacity	A	-	-	2	1	1	-	0	-	-	-	-	-
	Area involved	B	-	-	3	2	1	-	0	-	-	-	-	-
	(AxB) x 5		-	-	30	10	5	-	0	-	-	-	-	-
Iris		C	-	-	2	1	1	-	0	-	-	-	-	-
	C x 5		-	-	10	5	5	-	0	-	-	-	-	-
Conjunctiva	Redness	D	-	-	2	2	2	-	0	-	-	-	-	-
	Chemosis	E	-	-	2	1	1	-	0	-	-	-	-	-
	Discharge	F	-	-	2	1	1	-	0	-	-	-	-	-
	(D+E+F) x 2		-	-	12	8	8	-	0	-	-	-	-	-
<b>Total</b>			-	-	<b>52</b>	<b>23</b>	<b>18</b>	-	<b>0</b>	-	-	-	-	-

nf

Animal No.	5		Observation period (days)											
			1 h	4 h	1	2	3	4	7	9	10	12	14	21
Cornea	Opacity	A	-	-	2	1	1	-	1	-	1	-	1	1
	Area involved	B	-	-	4	2	1	-	1	-	1	-	1	1
	(AxB) x 5		-	-	40	10	5	-	5	-	5	-	5	5
Iris		C	-	-	2	1	1	-	0	-	0	-	2	0
	C x 5		-	-	10	5	5	-	0	-	0	-	10	0
Conjunctiva	Redness	D	-	-	3	2	2	-	2	-	1	-	1	0
	Chemosis	E	-	-	3	2	2	-	2	-	1	-	1	0
	Discharge	F	-	-	3	2	2	-	2	-	1	-	1	0
	(D+E+F) x 2		-	-	18	12	12	-	12	-	6	-	6	0
<b>Total</b>			-	-	<b>68</b>	<b>27</b>	<b>22</b>	-	<b>17</b>	-	<b>11</b>	-	<b>21</b>	<b>5</b>

Animal No.	6		Observation period (days)											
			1 h	4 h	1	2	3	4	7	9	10	12	14	21
Cornea	Opacity	A	-	-	2	2	2	-	0	-	0	-	0	-
	Area involved	B	-	-	4	2	1	-	0	-	0	-	0	-
	(AxB) x 5		-	-	40	20	10	-	0	-	0	-	0	-
Iris		C	-	-	2	1	1	-	1	-	0	-	0	-
	C x 5		-	-	10	5	5	-	5	-	0	-	0	-
Conjunctiva	Redness	D	-	-	3	2	2	-	1	-	1	-	0	-
	Chemosis	E	-	-	3	2	2	-	0	-	0	-	0	-
	Discharge	F	-	-	3	2	1	-	0	-	0	-	0	-
	(D+E+F) x 2		-	-	18	12	10	-	2	-	2	-	0	-
<b>Total</b>			-	-	<b>68</b>	<b>37</b>	<b>25</b>	-	<b>7</b>	-	<b>2</b>	-	<b>0</b>	-

MMAS (Modified Maximum Average Score) (54+47+66+52+68+68) / 6 = 59.2